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(54) Title: DETECTION OF RETROVIRAL SUBTYPES BASED UPON ENVELOPE SPECIFIC SEQUENCES

(57) Abstract

The present invention is based upon the finding that porcine endogenous retroviruses exist in two different subtypes, which we have termed PERV-A and PERV-B. The differences are reflected in sequence divergence in the envelope genes, and these differences may be used to provide nucleic acid and antibody probes which can distinguish between the two subtypes. This allows patterns of subtype transmission between cells, particularly porcine to human cells, to be monitored.

DETECTION OF RETROVIRAL SUBTYPES BASED UPON ENVELOPE SPECIFIC SEQUENCES

The present invention relates to methods and products for the detection of porcine endogenous retroviruses.

There is currently much interest in the development of xenotransplantation of organs to meet the shortage of human organs available for transplant. Considerable progress has been made in developing transgenic animals, particularly pigs, whose organs have been modified to remove immunogenic surface antigens and/or to present human antigen, or to inhibit components of the human immune system. However while progress has been made on the immunological problems of xenotransplantation, relatively little research has been conducted on the risk of infection being transmitted to an organ recipient by the presence of endogenous pathogens in the donor organ.

Recently, Patience *et al*, Nature Medicine, 1997, 3;282-286, reported the results of a study of pig endogenous retroviruses (PERVs) in porcine cell lines. The authors demonstrated that two different pig kidney cell lines, PK15 and MPK, produced endogenous retroviruses and the PK15 retroviruses were capable of infecting a human cell line (kidney 293 cells). Analysis of the protease and reverse transcriptase genes of the retroviruses infecting these cell lines showed that there was about 95% sequence similarity at the amino acid level between isolates from the two cell lines. This information was used to design nucleic acid primers for the analysis of DNA from porcine tissue and the authors demonstrated that multiple PERV related sequences existed in such tissue and were expressed. The primers were specific for porcine PERVs and did not detect sequences in human or murine cells.

WO97/21836, published on 19 June 1997, describes three porcine retrovirus isolates. These isolates are currently described as PERV-A and PERV-C, with SEQ ID NO:1 and SEQ ID NO:3 of

WO97/21836 being of the PERV-C type, and SEQ ID NO:2 being of the PERV-A type.

WO97/40167, published on 30 October 1997, describes a retrovirus isolate from the PK-15 porcine cell line. This isolate is currently described in the art as being of a PERV-B type. Figure 3 of WO97/40167 sets out a sequence with 3 open reading frames indicated to be the *gag*, *pol* and *env* genes of the retrovirus. Figure 1 of WO97/40167 sets out a shorter sequence with a 3' end which extends into the 5' region of the *env* gene. There are differences between the 3' end of Figure 1 and the corresponding region of Figure 3. The differences are attributed in WO97/40167 to improvements in carrying out and analysing the sequence obtained.

Disclosure of the invention.

Prior to the present invention, it had not been appreciated that PERVs existed in different subtypes. Prior to the publication of WO97/21836 and WO97/40167 we surprisingly identified two subtypes of this virus, which we designated PERV-A and PERV-B. More surprisingly, although the majority of individual isolates from the PK15 cell line are PERV-A isolates (29/32 tested), our initial data indicated that human 293 cells infected with the virus are exclusively or almost exclusively of the PERV-B subtype. Thus although the primers used by Patience *et al* are capable of detecting numerous PERV sequences in porcine tissue and cell lines, these primers do not distinguish between the two subtypes of PERV.

In the light of the present invention we believe that the sequence of Figure 1 of WO97/40167 is derived from a PERV-A isolate, since the Figure 1 sequence in the region of difference is substantially similar to the corresponding portion of the PERV-A isolate described herein.

In a first aspect the present invention thus provides an

isolated nucleic acid probe, said probe being capable of hybridising to the PERV-B *env* gene under conditions in which said probe is substantially unable to hybridise to the PERV-A *env* gene. This is referred to below as a PERV-B specific probe (or "primer" or "oligonucleotide"). The terms "probe", "primer" and "oligonucleotide" are used synonymously.

In a second aspect, the invention provides an isolated nucleic acid probe, said probe being capable of hybridising to the PERV-A *env* gene under conditions in which said probe is substantially unable to hybridise to the PERV-B *env* gene. This is referred to below as a PERV-A specific probe (or "primer" or "oligonucleotide").

Although the *env* gene sequences are shown as the positive strand, it is to be understood that probes of the invention may be directed to either strand where integrated or cDNA retroviral sequences are to be detected. Where retroviral RNA is to be detected, a probe capable of hybridising to the positive strand is required (in the case of PCR initially to make cDNA).

In a further aspect, the invention provides a pair of primers suitable for conducting a polymerase chain reaction, at least one of said primers being a nucleic acid as defined above specific for the PERV-A or PERV-B genes. The probes and primers of the invention may be used in a method of detecting retroviruses in a sample of porcine or human tissue. Such tissue includes primary porcine tissue and human cell lines which have been cultivated in the presence of a porcine cell line, or human tissues which are from a human patient who has received a xenotransplant. Nucleic acid (e.g. mRNA, total RNA, DNA or total nucleic acid) from the tissues or cells may be probed directly or if desired retroviral sequences may be amplified using primers suitable for amplifying retroviral sequences in general (e.g. LTR primers) prior to detecting PERV *env* sequences of the invention, thus allowing those of

skill in the art to distinguish between the PERV-A and PERV-B subtypes. The nucleic acid may be present in a sample comprising human or porcine tissue or cells, or may be cloned nucleic acid from such sources.

The differences between the two genes is reflected by changes to the *env* proteins, and these differences are believed to include differences to antigenic determinants (referred to herein as epitopes) in the two subtypes of proteins, which thus allows the development of antibodies which are capable of binding to an epitope on the PERV-B *env* protein under conditions where they are substantially unable to bind to the PERV-A *env* protein, and vice versa. These antibodies may be used in a method of detecting the presence of a pig endogenous retrovirus in porcine or human tissue or cell lines, thus allowing those of skill in the art to distinguish between the PERV-A and PERV-B subtypes.

<u>Detailed Description of the Invention.</u>

Our prototype isolate of the PERV-A *env* gene region is shown in SEQ ID NO. 1, and the envelope polypeptide encoded by nucleotides 211 to 2190 of SEQ ID NO. 1 is shown as SEQ ID NO. 2. For the purposes of the present invention, the PERV-A *env* gene is at least 80%, preferably at least 90% and more preferably at least 95% homologous to the coding sequence of SEQ ID NO. 1. Homologous sequences include those which encode the same polypeptide shown in SEQ ID NO:2 but differ from SEQ ID NO:1 due to the degeneracy of the genetic code.

The percentage homology (also referred to as identity) of DNA sequences can be calculated using commercially available algorithms, such as Lasergene software from DNASTAR Inc or the algorithm GAP (Genetics Computer Group, Madison, WI). GAP uses the Needleman and Wunsch algorithm to align two complete sequences that maximizes the number of matches and minimizes the number of gaps. Generally, the default parameters are

used, with a gap creation penalty = 12 and gap extension penalty = 4. Use of either of the terms "homology" and "homologous" herein does not imply any necessary evolutionary relationship between compared sequences, in keeping for example with standard use of terms such as "homologous recombination" which merely requires that two nucleotide sequences are sufficiently similar to recombine under the appropriate conditions.

Similarly, our prototype isolate of the PERV-B *env* gene region is shown in SEQ ID NO. 3, and the envelope polypeptide encoded by nucleotides 911 to 2881 of SEQ ID NO. 3 is shown as SEQ ID NO. 4. For the purposes of the present invention, the PERV-B *env* gene is at least 80%, preferably at least 90% and more preferably at least 95% homologous to the coding sequence of SEQ ID NO. 3. Homologous sequences include those which encode the same polypeptide shown in SEQ ID NO:4 but differ from SEQ ID NO:3 due to the degeneracy of the genetic code.

An alignment of SEQ ID NO. 1 and SEQ ID NO. 3 is shown as Figure 1.

The PERV-B specific probe of the invention is preferably derived from the 5′ end of the *env* gene of PERV-B, particularly from the region of PERV-B corresponding to nucleotides 1000 to 2500 of the SEQ ID NO. 3 isolate. More preferably the region corresponds to nucleotides 1100 to 1900.

It is to be understood that "derived" means conceptually derived, and physical isolation of the nucleic acid from the gene (as opposed to, for example, de novo synthesis) is not necessary.

Specific PERV-B probes include oligonucleotides consisting of a contiguous sequence of from 10 to 40 nucleotides of a PERV-B isolate derived from the sequence of SEQ ID NO:3 from 1000 to 2500, preferably 1100 to 1900, or the complement thereof.

Such oligonucleotides include SEQ ID NO:7 (1376-1395 of SEQ ID NO:3) and SEQ ID NO:8 (complement of 1620-1639 of SEQ ID NO:3) shown in Example 3 below comprise 8 and 14 differences respectively in their sequences and the corresponding regions of SEQ ID NO:1 as follows:

```
PERV-B     5' TTCTCCTTTGTCAA--TTCCGG 3'  (SEQ ID NO:7)
               *   *     *  ***   **
PERV-A     5' TACTCTTTTGTTAACAATCCTA 3'  (SEQ ID NO:9)
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and:

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PERV-B     5' TACTTTATCGGGTCCCACTG 3'  (SEQ ID NO:8)
                * * *** * ********
PERV-A     5' TATTCTGAGGCGCGAATAGT 3'  (SEQ ID NO:10)
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Similarly, the PERV-A specific probe of the invention may be derived from the regions shown in Figure 1 which correspond to the abovementioned preferred and most preferred regions of PERV-A. Thus PERV-A specific probes include oligonucleotides consisting of a contiguous sequence of from 10 to 40 nucleotides of a PERV-A isolate derived from the sequence of SEQ ID NO:1 from 300 to 1809, preferably 400 to 1209, or the complement thereof.

Thus for example such oligonucleotides include SEQ ID NO:5 (742-760 of SEQ ID NO:1) and SEQ ID NO:6 (complement of 1082-1101 of SEQ ID NO:1) shown in Example 3 below. These comprise 10 and 21 differences respectively in their sequences and the corresponding regions of SEQ ID NO:3.

By "differences", it is meant substitutions, deletions and insertions. As can be seen from Figure 1, the primers of SEQ ID NOs:5-8 include between them all these differences from the corresponding portions of the reference isolate.

The above-mentioned probes may additionally include, at their

3′ and/or 5′ termini, linker sequences (typically of from 3 to 8 nucleotides) of non-PERV-B or -A sequence. Linker sequences include those containing a restriction enzyme recognition sequence allowing the oligonucleotides to be introduced into or excised from a cloning or expression vector.

Nucleic acid probes of the invention may be obtained by first of all comparing the PERV-A and PERV-B sequences of Figure 1 (or of other PERV-A and PERV-B isolates) and regions of the sequences which are sufficiently different to provide specific probes determined. This may be done by any suitable means, for example by calculating the predicted Tm of a probe when annealed to a specific region of the PERV-A or PERV-B sequences using a suitable algorithm or empirically by experiment. When by experiment this can be achieved by blotting the PERV-A and PERV-B sequences onto a nitrocellulose filter and probing the filter with a labelled putative probe under hybridising conditions. Probes of the invention will be able to hybridise to the PERV sequence of choice and not to the other PERV sequence under those conditions. Thus a PERV-B specific probe of the invention will be capable of hybridising to the sequence of SEQ ID NO:3 under conditions in which the probe does not hybridise to SEQ ID NO:1. Similarly, a PERV-A specific probe of the invention will be capable of hybridising to the sequence of SEQ ID NO:1 under conditions in which it does not hybridise to SEQ ID NO:3.

Hybridisation conditions will be selected to be commensurate with the size of the probe and can be determined by reference to standard text books such as Sambrook et al, Molecular Cloning, 1989, Cold Spring Harbour.

It will be understood by those of skill in the art that hybridisation conditions will vary depending upon whether a probe of the invention is hybridised to nucleic acid fixed to a solid support or is hybridised to a target nucleic acid in a liquid phase. In the case of the former (eg Southern or

Northern blotting) a probe of the invention will be annealed under low stringency conditions and subsequently washed under high stringency conditions such that the probe will remain annealed to its target PERV sequence and not to the corresponding sequence of the other subtype. Where a probe of the invention is for use as a PCR primer annealing conditions will be selected in accordance with standard protocols such that the probe will hybridise to its target subtype nucleic acid and not to non-target subtype nucleic acid. Thus it will be understood that reference to hybridisation of a probe to target nucleic acid includes hybridisation achieved by blotting and washing on a solid phase as well as annealing in a liquid phase. In either case, the person of skill in the art will be able to test using routine skill and knowledge whether any selected sequence derived from a PERV-B *env* gene is able to hybridise to the PERV-B *env* nucleic acid under conditions in which it is substantially unable to hybridise to PERV-A *env* nucleic acid, and vice versa.

One way to calculate Tm of a probe is by reference to the formula for calculating the Tm of probes to a homologous target sequence. This formula is Tm(°C) = 2(A+T) + 4(G+C) - 5. This will provide the Tm under conditions of 3xSSC and 0.1% SDS (where SSC is 0.15M NaCl, 0.015M sodium citrate. pH 7). This formula is generally suitable for probes of up to 30 nucleotides in length. In the present invention, this formula may be used as an algorithm to calculate a nominal Tm of a probe for a specified sequence based upon the number of matches to its PERV target (e.g. PERV-B) sequence and PERV non-target sequence (e.g. PERV-A). For example, for the probe of SEQ ID NO:7 has a Tm of ((2x11) + (4x9) - 5) = 53°C. The sequence of SEQ ID NO:7 is derived from SEQ ID NO:3 and thus will have this Tm when used as a probe for this sequence, subject to the usual experimental error. However when SEQ ID NO:7 is used as a probe for the corresponding region of SEQ ID NO:1 (represented above as SEQ ID NO:9), the calculated Tm will be ((2x9) + (4x5) - 5) = 33°C, based on counting the

number of matches. (Since for the purposes of the present invention the above formula is used as an algorithm, the actual Tm of probes when hybridised to non-complementary targets which do not exactly match the probe sequence may or may not correspond to the calculated value.)

Thus in a preferred aspect, a PERV-B specific probe will have a Tm (calculated as above) for SEQ ID NO:3 which is at least 5°C higher than for SEQ ID NO:1, and vice versa for a PERV-A specific probe. Preferably the difference is at least 8°C, more preferably at least 10°C, at least 15°C or at least 20°C.

The above formula generally useful for probes up to 30 nucleotides in length, but since it is used simply as an algorithm in the present invention, it may be extended to longer probes, for example up to 40 or even up to 50 nucleotides in length.

Suitable conditions for a probe to hybridise to a PERV target sequence may also be measured experimentally. Suitable experimental conditions comprise hybridising a candidate probe to both SEQ ID NO:1 and SEQ ID NO:3 on a solid support under low stringency hybridising conditions (e.g. 6xSSC at 55°C), washing at reduced SSC and/or higher temperature, for example at 0.2xSSC at 45°C, and increasing the hybridisation temperature incrementally to determine hybridisation conditions which allow the probe to hybridise to SEQ ID NO:1 but not SEQ ID NO:3, or vice versa, as the case may be.

Although the hybridisation conditions used to distinguish between the PERV-B and PERV-A *env* genes should also be sufficient to distinguish over other "background" sequences present in human or porcine cells (particularly human and porcine genomic and mitochondrial sequences), it is also desirable that the probes do not, under such conditions, hybridise to such background sequences. This may also be determined by experiment, for example by blotting the probes

to a solid support which carries at separate loci SEQ ID NO:1, SEQ ID NO:3 (for example cloned in plasmids), human total DNA and porcine total DNA.

The size of the probe may be selected by those of skill in the art taking account of the particular purposes the probes are to be used. Probes may be for example from 10 to 1000 nucleotides (or base pairs), e.g. from 50 to 500, such as from 200 to 500 nucleotides or base pairs. This size range is particularly suitable for Southern blots. However for some purposes, for example PCR, short oligonucleotide probes are preferred, generally in the size range of from 10 to 40 nucleotides in length, preferably 12 to 25 and more preferably from 18 to 24 such as 20, 21 or 22 nucleotides.

The probes may be labelled with a detectable label, including a radionuclide such as ^{32}P or ^{35}S which can be added to the probe using methods known per se in the art. The probe may alternatively carry a non-radioactive label such as biotin.

Generally, probes will be prepared by stepwise chemical synthesis, which is widely available commercially. Recombinant production of probes is also possible. Probes may be DNA or RNA, and may contain or consist of synthetic or modified nucleotides. A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothionate backbones, addition of acridine or polylysine chains at the 3′ and/or 5′ ends of the molecule. For the purposes of the present invention, it is to be understood that the probes and primers described herein may be modified by any method available in the art.

A preferred method of detection is by the polymerase chain reaction (PCR). This will involve PERV-B or PERV-A primer pairs, at least one of which is directed to PERV-B or PERV-A *env* gene sequences, the polarity of the probes being such that

the region between them is amplified when the PCR is performed. At least one of each pair of PERV-A and/or PERV-B primers will be specific for its target PERV sequence. The other member of each pair may be targeted to non-*env* sequence or *env* sequence common to PERV-A and PERV-B. Preferably both members of a primer pair are specific for their target PERV sequence. Desirably the probes will be selected to amplify a region of the PERV-A and PERV-B of a convenient size to detect, such as between about 50 and 500, preferably between 150 and 400 nucleotides.

Where pairs of PERV-A and PERV-B primers are used in conjunction with each other, it is preferred that the primer pairs are selected such that different size PERV-A and PERV-B products are produced. Preferably the difference in size is at least from 5 to 50 base pairs, such as from 10 to 25 base pairs, so that detection of the products by electrophoresis on agarose gels by ethidium bromide staining may be conveniently carried out.

The methods of the invention which allow the PERV-A and PERV-B subtypes to be distinguished are useful in following the transmission of these viruses from porcine cells to other cell types, particularly human cells. In addition, the probes may be used to clone and characterize the different endogenous proviruses of pigs. Specific proviruses can be characterised by both their sequences and the genomic flanking sequences, and thus a map of the chromosomal locations of the viruses may be determined. The ability to distinguish between PERV-A and PERV-B proviruses will facilitate studies of the porcine endogenous retroviruses which might pose a threat to humans in a transplant setting.

The PERV-A and PERV-B nucleic acid sequences of the invention are novel and thus in a further aspect of the invention there is provided an isolated nucleic acid consisting essentially of the PERV-A or PERV-B *env* gene coding sequence, or a fragment

thereof which is capable of hybridising to the PERV-B *env* gene under conditions in which said probe is substantially unable to hybridise to the PERV-A *env* gene, or vice versa. Vectors which comprise such sequences form a further aspect of the invention. The vector may be for replication of the sequence or for expression of the sequence in a suitable host cell. In such a case the vector will comprise a promoter operably linked to the *env* sequence, the promoter being compatible with the host cell which may be, for example, bacterial, e.g. *E.coli*, yeast, insect or mammalian, e.g. a CHO cell or a human cell line.

The *env* gene may be expressed in such a cell and recovered from the cell in substantially isolated form.

The differences in the PERV subtypes also allow the production of antibodies which can distinguish between the two subtypes. In a manner analogous to the production of probes, the sequence differences between the proteins of SEQ ID NO. 2 and SEQ ID NO. 4 can be examined, and suitable epitopes which reflect these differences determined using computer algorithms or by epitope scanning techniques. Monoclonal antibodies raised against these epitopes may be used to detect the presence of the PERV-A and/or PERV-B subtypes in a specific manner.

In a manner analogous to the nucleic acid probes, the antibodies are preferably directed to epitopes in the N-terminal region of the PERV-A and PERV-B *env* proteins, particularly epitopes encoded within the preferred regions identified above.

For the purposes of the present invention the term antibody describes an immunoglobulin whether natural or partly or wholly synthetically produced. The term also covers any polypeptide or protein having a binding domain which is, or is homologous to, an antibody binding domain. These can be

derived from natural sources, or they may be partly or wholly synthetically produced. Examples of antibodies are the immunoglobulin isotypes and their isotypic subclasses; fragments which comprise an antigen binding domain such as Fab, scFv, Fv, dAb, Fd; and diabodies.

It is possible to take monoclonal and other antibodies and use techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP-A-184187, GB 2188638A or EP-A-239400. A hybridoma or other cell producing an antibody may be subject to genetic mutation or other changes, which may or may not alter the binding specificity of antibodies produced.

As antibodies can be modified in a number of ways, the term "antibody" should be construed as covering any specific binding member or substance having a binding domain with the required specificity. Thus, this term covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including any polypeptide comprising an immunoglobulin binding domain, whether natural or wholly or partially synthetic. Chimeric molecules comprising an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023.

It has been shown that fragments of a whole antibody can perform the function of binding antigens. Examples of binding fragments are (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH

domains of a single antibody; (iv) the dAb fragment (Ward, E.S. et al., Nature 341, 544-546 (1989)) which consists of a VH domain; (v) isolated CDR regions; (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al, Science, **242**, 423-426, 1988; Huston et al, PNAS USA, **85**, 5879-5883, 1988); (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; P. Holliger et al Proc. Natl. Acad. Sci. USA **90** 6444-6448, 1993).

The reactivities of antibodies to an epitope in a sample may be determined by any appropriate means. Tagging with individual reporter molecules is one possibility. The reporter molecules may directly or indirectly generate detectable, and preferably measurable, signals. The linkage of reporter molecules may be directly or indirectly, covalently, eg via a peptide bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding antibody and reporter molecule.

One favoured mode is by covalent linkage of each antibody with an individual fluorochrome, phosphor or laser dye with spectrally isolated absorption or emission characteristics. Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red. Suitable chromogenic dyes include diaminobenzidine. Other reporters include macromolecular colloidal particles or particulate material such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or otherwise recorded. These molecules may be enzymes which catalyse

reactions that develop or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions between energy states result in characteristic spectral absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase detection systems may be employed.

A radionuclide such as ^{125}I, ^{111}In or ^{99m}Tc may be attached to an antibody and these nuclides are useful in imaging target antigens in the body. Antibodies labelled with these labels may be used to examine xenotransplanted organs in a human recipient for the presence of PERVs as part of ongoing monitoring following transplantation.

Antibodies of the invention may be produced by conventional hybridoma technology, e.g by linking a peptide comprising a suitable epitope to a carrier protein, injecting the linked peptide into an animal such as a rat or rabbit, recovering the spleen and producing hybridoma cell lines which are screened against the peptide for specific binding. Antibodies may also be prepared by screening against synthetic libraries such as phage display libraries. Antibodies may also be made against the entire *env* protein or substantial parts thereof, and then screened individually against PERV-A and PERV-B *env* protein for specific binding to one or the other.

In one aspect of the invention a specific PERV-A antibody and a specific PERV-B antibody are used on parallel samples (or on the same sample where the two antibodies are labelled with different and distinguishable labels) to detect the presence of the two subtypes of retroviruses.

Antibodies specific for a PERV-B epitope will have at least a 100 fold higher affinity for that epitope than for the corresponding region (as indicated by alignments to the PERV-A

sequence such as that of Figure 1) of the PERV-A *env* protein., and vice versa. Desirably both types of specific antibodies will not cross react to other proteins normally present in human and porcine cells (i.e. have at least a 100 fold higher affinity to its target epitope than to such other proteins).

The probes, primers and antibodies of the invention may be used in all aspects of the development of porcine organ (e.g. kidney, liver, heart, pancreas, including tissues and cells therefrom, such as pancreatic islet cells) xenotrans-plantation. Thus the probes, primers and antibodies may be used to monitor the inheritance of human tropic viruses, thus facilitating the breeding of pigs lacking these viruses, particularly the PERV-B subtype. The invention will also be useful in monitoring the expression of the viruses in pigs and humans.

The following examples illustrate the invention.

Example 1: Cloning of PERV-A and PERV-B *Env* sequences.

cDNA clones were obtained using the 3′ RACE technique (Frohman and Martin Technique 1:165-170, 1989). Total RNA from PK15, MPK and 293 cells was reverse transcribed to produce cDNA using an adapter primer dT-Ri-Ro.

A fraction of cDNA from PK15 and MPK cells was amplified by the polymerase chain reaction (PCR) using the primer PL146 (5′ATCCGTCGGCATGCATAATACGACTCAC, SEQ ID NO:11) in combination with PL135 (5′CGATTCAGTGCTGCTACAAC, SEQ ID NO:12) or PL137 (5′CCCTTATAACCTCTTGAGCG, SEQ ID NO:13). Products of approximately 6.5 kb were digested with *Xho*I and *Sph*I and cloned into *Sal*I//*Sph*I digested pGem3Zf(+). Positive clones were identified and sequenced.

A portion of cDNA from 293 cells was amplified by PCR using primer PL137 in combination with primer Ro. Products of

approximately 6.5 kb were isolated and digested with *Pst*I and ligated with the pGem3Zf(+) plasmid digested with *Pst*I and *Sma*I. After transformation into *E.coli*, positive clones were identified and sequenced.

Further clones were generated and sequenced from MPK and PK15 cDNA by amplification with primer PL147 (5'GTAATGCATGCTTCTATGGTGCCAGTCG, SEQ ID NO:14) in combination with either PL135, PL137 or PL148 (5'CTCTACGCATGCGTGGTGTACGACTGTG, SEQ ID NO:15) and digestion of products with *Xho*I/*Sph*I or *Sph*I and cloning into appropriately digested pGEM3Zf(+).

Further clones were generated and sequenced from 293 cDNA by PCR amplification with primer PL147 in combination with either PL135, PL137 or PL149 (5'GTAATCGGGTCAGACAATGG, SEQ ID NO:16) and digestion of products with *Eco*RI/*Pst*I, *Pst*I, or *Bam*HI/*Eco*RI and cloning into appropriately digested pGem3Zf(+).

Oligos dT-Ri-Ro and Ro come from Frohman and Martin (Technique 1:165-170,1989), PL146 is a modified version of Ro containing an additional SphI site, PL135 and PL137 were designed from the published PERV pol sequence (Tristan *et al* J.Virol 70:8241-8246, 1996 Genbank ID X99933), PL147 and PL148 are PERV LTR primers derived from the sequences of our initial 293 clones.

Analysis of the clones identified two distinct subtypes, which we have termed PERV-A and PERV-B. An alignment of the two subtype envelope gene sequences is shown in Figure 1.

<u>Example 2: Frequency of full length PERV-A and PERV-B *env* gene isolation.</u>

The frequency of the subtypes in pig and human cells was analysed and the results are as follows:

1. From pig PK-15 cells
   29/32 PERV-A   3/32 PERV-B

2. From human 293 cells infected with PK15 virus
   0/18 PERV-A   18/18 PERV-B

Example 3: Preparation of specific probes

1. PCR

Differences between the PERV-A and PERV-B subgroups allow the design of specific primers

PL170 TGGAAAGATTGGCAACAGCG (SEQ ID NO:5)
PL171 AGTGATGTTAGGCTCAGTGG (SEQ ID NO:6)
PL172 TTCTCCTTTGTCAATTCCGG (SEQ ID NO:7)
PL173 TACTTTATCGGGTCCCACTG (SEQ ID NO:8)

PL170+PL171 are predicted to give a 361 base pair band with PERV-A;

PL172+PL173 are predicted to give a 264 base pair band with PERV-B. PCR studies with cloned plasmid DNA confirmed these prediction and showed no cross-amplification between the two primer pairs. Sequencing the respective RT-PCR products from RNA containing both viral RNAs shows amplification only of the sequences predicted from each primer pair.

2. Southern blot probes.

The amplification products of PL170+PL171 (361 bp, PERV-A probe) and PL172+PL173 (264 bp, PERV-B probe) show no cross hybridisation on plasmid blots. Both have been used on genomic southern blots.

Example 4: Host range studies

The host range specified by the cloned PERV *env* genes were examined using a Moloney murine leukemia virus (Mo-MLV) based vector to deliver the β-galactosidase (lacZ) indicator gene to different cell types (Tailor *et al* J.Virol. 67:6737-6741,

1993). The TELCeB6 cell line (Cosset *et al* J.Virol. 69: 7430-7436, 1995) is derived from TE671 cells by stable transfection with CeB to supply the Mo-MLV gag-pol genes and carrying a modified lacZ gene (Ferry *et al* PNAS 88: 8377-8381, 1991) in proviral context introduced by infection using an amphotropic viral vector. The PERV *env* genes were introduced by transfection of TELCeB6 cells with expression constructs derived from pFBMOSALF (Cosset *et al* J.Virol, 69: 6314-6322, 1995) in which the PERV sequences, on XbaI-ClaI fragments, replace the corresponding Mo-MLV envelope sequence. Virus produced by transiently and stably transfected TELCeB6 cells were assayed for transfer of LacZ on 293, TE671 (human) and PK-15, PAE, ST-IOWA (pig) cells. Transfer of retroviral particles comprising the PERV-B envelope to human cells was demonstrated.

The infectious titre (LacZ positives/ml supernatant) was as follows:

Virus	Pig (ST-IOWA)	Mink (Mu-1-lv)	Human (293)	Human (TE671)
PERV-A	2000	1000	300	2000
PERV-B	800	4000	800	700

SEQUENCE LISTING

SEQ ID NO. 1: PERV-A.seq

TCGAGTGGGT GAGGCAGCGA GCGTGGAAGC AGCTCCGGGA GGCCTACTCA
GGAGGAGACT TGCAAGTTCC ACATCGCTTC CAAGTTGGAG ATTCAGTCTA 100
TGTTAGACGC CACCGTGCAG GAAACCTCGA GACTCGGTGG AAGGGACCTT
ATCTCGTACT TTTGACCACA CCAACGGCTG TGAAAGTCGA AGGAATCCCC 200
ACCTGGATCC ATGCATCCCA CGTTAAGCCG GCGCCACCTC CCGATTCGGG
GTGGAAAGCC GAAAAGACTG AAAATCCCCT TAAGCTTCGC CTCCATCGCG 300
TGGTTCCTTA CTCTGTCAAT AACTCCTCAA GTTAATGGTA AACGCCTTGT
GGACAGCCCG AACTCCCATA AACCCTTATC TCTCACCTGG TTACTTACTG 400
ACTCCGGTAC AGGTATTAAT ATTAACAGCA CTCAAGGGGA GGCTCCCTTG
GGGACCTGGT GGCCTGAATT ATATGTCTGC CTTCGATCAG TAATCCCTGG 500
TCTCAATGAC CAGGCCACAC CCCCCGATGT ACTCCGTGCT TACGGGTTTT
ACGTTTGCCC AGGACCCCCA AATAATGAAG AATATTGTGG AAATCCTCAG 600
GATTTCTTTT GCAAGCAATG GAGCTGCATA ACTTCTAATG ATGGGAATTG
GAAATGGCCA GTCTCTCAGC AAGACAGAGT AAGTTACTCT TTTGTTAACA 700
ATCCTACCAG TTATAATCAA TTTAATTATG GCCATGGGAG ATGGAAAGAT
TGGCAACAGC GGGTACAAAA AGATGTACGA AATAAGCAAA TAAGCTGTCA 800
TTCGTTAGAC CTAGATTACT TAAAAATAAG TTTCACTGAA AAAGGAAAAC
AAGAAAATAT TCAAAAGTGG GTAAATGGTA TATCTTGGGG AATAGTGTAC 900
TATGGAGGCT CTGGGAGAAA GAAAGGATCT GTTCTGACTA TTCGCCTCAG
AATAGAAACT CAGATGGAAC CTCCGGTTGC TATAGGACCA AATAAGGGTT 1000
TGGCCGAACA AGGACCTCCA ATCCAAGAAC AGAGGCCATC TCCTAACCCC
TCTGATTACA ATACAACCTC TGGATCAGTC CCCACTGAGC CTAACATCAC 1100
TATTAAAACA GGGGCGAAAC TTTTTAGCCT CATCCAGGGA GCTTTTCAAG
CTCTTAACTC CACGACTCCA GAGGCTACCT CTTCTTGTTG GCTTTGCTTA 1200
GCTTCGGGCC CACCTTACTA TGAGGGAATG GCTAGAGGAG GGAAATTCAA
TGTGACAAAG GAACATAGAG ACCAATGTAC ATGGGGATCC CAAAATAAGC 1300
TTACCCTTAC TGAGGTTTCT GGAAAAGGCA CCTGCATAGG GATGGTTCCC
CCATCCCACC AACACCTTTG TAACCACACT GAAGCCTTTA ATCGAACCTC 1400
TGAGAGTCAA TATCTGGTAC CTGGTTATGA CAGGTGGTGG GCATGTAATA
CTGGATTAAC CCCTTGTGTT TCCACCTTGG TTTTCAACCA AACTAAAGAC 1500
TTTTGCGTTA TGGTCCAAAT TGTCCCCCGG GTGTACTACT ATCCCGAAAA
AGCAGTCCTT GATGAATATG ACTATAGATA TAATCGGCCA AAAAGAGAGC 1600
CCATATCCCT GACACTAGCT GTAATGCTCG GATTGGGAGT GGCTGCAGGC
GTGGGAACAG GAACGGCTGC CCTAATCACA GGACCGCAAC AGCTGGAGAA 1700
AGGACTTAGT AACCTACATC GAATTGTAAC GGAAGATCTC CAAGCCCTAG

AAAAATCTGT CAGTAACCTG GAGGAATCCC TAACCTCCTT ATCTGAAGTG 1800
GTTCTACAGA ACAGAAGGGG GTTAGATCTG TTATTTCTAA AAGAAGGAGG
GTTATGTGTA GCCTTAAAAG AGGAATGCTG CTTCTATGTA GATCACTCAG 1900
GAGCCATCAG AGACTCCATG AGCAAGCTTA GAGAAAGGTT AGAGAGGCGT
CGAAGGGAAA GAGAGGCTGA CCAGGGGTGG TTTGAAGGAT GGTTCAACAG 2000
GTCTCCTTGG ATGACCACCC TGCTTTCTGC TCTGACGGGG CCCCTAGTAG
TCCTGCTCCT GTTACTTACA GTTGGGCCTT GCTTAATTAA TAGGTTTGTT 2100
GCCTTTGTTA GAGAACGAGT GAGTGCAGTC CAGATCATGG TACTTAGGCA
ACAGTACCAA GGCCTTCTGA GCCAAGGAGA AACTGACCTC TAGCCTTCCC 2200
AGTTCTAAGA TTAGAACTAT TAACAAGACA AGAAGTGGGG AATGAAAGGA
TGAAAATGCA ACCTAACCCT CCCAGAACCC AGGAAGTTAA TAAAAAGCTC 2300
TAAATGCCCC CGAATTCCAG ACCCTGCTGG CTGCCAGTAA ATAGGTAGAA
GGTCACACTT CCTATTGTTC CAGGGCCTGC TATCCTGGCC TAAGTAAGAT 2400
AACAGGAAAT GAGTTGACTA ATCGCTTATC TGGATTCTGT AAAACCGACT
GGCACCATAG AA 2462

SEQ ID NO. 2: Translation of PERV-A *env* (1 letter code)

MHPTLSRRHLPIRGGK PKRLKIPLSFASIAWF LTLSITPQVNGKRLVD 48
SPNSHKPLSLTWLLTD SGTGININSTQGEAPL GTWWPELYVCLRSVIP 96
GLNDQATPPDVLRAYG FYVCPGPPNNEEYCGN PQDFFCKQWSCITSND 144
GNWKWPVSQQDRVSYS FVNNPTSYNQFNYGHG RWKDWQQRVQKDVRNK 192
QISCHSLDLDYLKISF TEKGKQENIQKWVNGI SWGIVYYGGSGRKKGS 240
VLTIRLRIETQMEPPV AIGPNKGLAEQGPPIQ EQRPSPNPSDYNTTSG 288
SVPTEPNITIKTGAKL FSLIQGAFQALNSTTP EATSSCWLCLASGPPY 336
YEGMARGGKFNVTKEH RDQCTWGSQNKLTLTE VSGKGTCIGMVPPSHQ 384
HLCNHTEAFNRTSESQ YLVPGYDRWWACNTGL TPCVSTLVFNQTKDFC 432
VMVQIVPRVYYYPEKA VLDEYDYRYNRPKREP ISLTLAVMLGLGVAAG 480
VGTGTAALITGPQQLE KGLSNLHRIVTEDLQA LEKSVSNLEESLTSLS 528
EVVLQNRRGLDLLFLK EGGLCVALKEECCFYV DHSGAIRDSMSKLRER 576
LERRRREREADQGWFE GWFNRSPWMTTLLSAL TGPLVVLLLLLTVGPC 624
LINRFVAFVRERVSAV QIMVLRQQYQGLLSQG ETDL* 660

SEQ ID NO. 3: PERV-B.seq

GCATGCCTGC AGCAGTTGGT CAGAACATCC CCTTATCATG TTCTGAGGCT
ACCAGGAGTG GCTGACTCGG TGGTCAAACA TTGTGTGCCC TGCCAGCTGG 100

TTAATGCTAA TCCTTCCAGA ATACCTCCAG GAAAGAGACT AAGGGGAAGC
CACCCAGGCG CTCACTGGGA AGTGGACTTC ACTGAGGTAA AGCCGGCTAA 200
ATACGGAAAC AAATATCTAT TGGTTTTTGT AGACACCTTT TCAGGATGGG
TAGAGGCTTA TCCTACTAAG AAAGAGACTT CAACCGTGGT GGCTAAAAAA 300
ATACTGGAGG AAATTTTTCC GAGATTTGGA ATACCTAAGG TAATCGGGTC
AGACAATGGT CCAGCTTTTG TTGCCCAGGT AAGTCAGGGA CTGGCCAAGA 400
TATTGGGGAT TGATTGGAAA CTGCATTGTG CATACAGACC CCAAAGCTCA
GGACAGGTAG AGAGGATGAA TAGAACCATT AAAGAGACCC TTACCAAATT 500
GACCACAGAG ACTGGCATTA ATGATTGGAT AGCTCTCCTG CCCTTTGTGC
TTTTTAGGGT TAGGAACACC CCTGGACAGT TTGGGCTGAC CCCCTATGAA 600
TTGCTCTACG GGGGACCCCC CCCGTTGGTA GAAATTGCTT CTGTACATAG
TGCTGATGTG CTGCTTTCCC AGCCTCTGTT CTCTAGGCTC AAGGCGCTCG 700
AGTGGGTGAG GCAACGAGCG TGGAAGCAGC TCCGGGAGGC CTACTCAGGA
GAAGGAGACT TGCAAGTTCC ACATCGCTTC CAAGTGGGAG ATTCAGTCTA 800
TGTTAGACGC CACCGTGCAG GAAACCTCGA GACTCGGTGG AAGGGCCCTT
ATCTCGTACT TTTGACCACA CCAACGGCTG TGAAAGTCGA AGGAATCTCC 900
ACCTGGATCC ATGCATCCCA CGTTAAGCTG GCGCCACCTC CCGACTCGGG
GTGGAGAGCC GAAAAGACTG AGAATCCCCT TAAGCTTCGC CTCCATCGCC 1000
TGGTTCCTTA CTCTAACAAT AACTCCCCAG GCCAGTAGTA AACGCCTTAT
AGACAGCTCG AACCCCCATA GACCTTTATC CCTTACCTGG CTGATTATTG 1100
ACCCTGATAC GGGTGTCACT GTAAATAGCA CTCGAGGTGT TGCTCCTAGA
GGCACCTGGT GGCCTGAACT GCATTTCTGC CTCCGATTGA TTAACCCCGC 1200
TGTTAAAAGC ACACCTCCCA ACCTAGTCCG TAGTTATGGG TTCTATTGCT
GCCCAGGCAC AGAGAAAGAG AAATACTGTG GGGGTTCTGG GGAATCCTTC 1300
TGTAGGAGAT GGAGCTGCGT CACCTCCAAC GATGGAGACT GGAAATGGCC
GATCTCTCTC CAGGACCGGG TAAAATTCTC CTTTGTCAAT TCCGGCCCGG 1400
GCAAGTACAA AGTGATGAAA CTATATAAAG ATAAGAGCTG CTCCCCATCA
GACTTAGATT ATCTAAAGAT AAGTTTCACT GAAAAAGGAA AACAGGAAAA 1500
TATTCAAAAG TGGATAAATG GTATGAGCTG GGGAATAGTT TTTTATAAAT
ATGGCGGGGG AGCAGGGTCC ACTTTAACCA TTCGCCTTAG GATAGAGACG 1600
GGGACAGAAC CCCCTGTGGC AGTGGGACCC GATAAAGTAC TGGCTGAACA
GGGGCCCCCG GCCCTGGAGC CACCGCATAA CTTGCCGGTG CCCCAATTAA 1700
CCTCGCTGCG GCCTGACATA ACACAGCCGC CTAGCAACGG TACCACTGGA
TTGATTCCTA CCAACACGCC TAGAAACTCC CCAGGTGTTC CTGTTAAGAC 1800
AGGACAGAGA CTCTTCAGTC TCATCCAGGG AGCTTTCCAA GCCATCAACT
CCACCGACCC TGATGCCACT TCTTCTTGTT GGCTTTGTCT ATCCTCAGGG 1900
CCTCCTTATT ATGAGGGGAT GGCTAAAGAA GGAAAATTCA ATGTGACCAA

AGAGCATAGA AATCAATGTA CATGGGGGTC CCGAAATAAG CTTACCCTCA 2000
CTGAAGTTTC CGGGAAGGGG ACATGCATAG GAAAAGCTCC CCCATCCCAC
CAACACCTTT GCTATAGTAC TGTGGTTTAT GAGCAGGCCT CAGAAAATCA 2100
GTATTTAGTA CCTGGTTATA ACAGGTGGTG GGCATGCAAT ACTGGGTTAA
CCCCCTGTGT TTCCACCTCA GTCTTCAACC AATCCAAAGA TTTCTGTGTC 2200
ATGGTCCAAA TCGTCCCCCG AGTGTACTAC CATCCTGAGG AAGTGGTCCT
TGATGAATAT GACTATCGGT ATAACCGACC AAAAAGAGAA CCCGTATCCC 2300
TTACCCTAGC TGTAATGCTC GGATTAGGGA CGGCCGTTGG CGTAGGAACA
GGGACAGCTG CCCTGATCAC AGGACCACAG CAGCTAGAGA AAGGACTTGG 2400
TGAGCTACAT GCGGCCATGA CAGAAGATCT CCGAGCCTTA GAGGAGTCTG
TTAGCAACCT AGAAGAGTCC CTGACTTCTT TGTCTGAAGT GGTTCTACAG 2500
AACCGGAGGG GATTAGATCT GCTGTTTCTA AGAGAAGGTG GGTTATGTGC
AGCCTTAAAA GAAGAATGTT GCTTCTATGT AGATCACTCA GGAGCCATCA 2600
GAGACTCCAT GAGCAAGCTT AGAGAAAGGT TAGAGAGGCG TCGAAGGGAA
AGAGAGGCTG ACCAGGGGTG GTTTGAAGGA TGGTTCAACA GGTCTCCTTG 2700
GATGACCACC CTGCTTTCTG CTCTGACGGG ACCCCTAGTA GTCCTGCTCC
TGTTACTTAC AGTTGGGCCT TGCTTAATTA ATAGGTTTGT TGCCTTTGTT 2800
AGAGAACGAG TGAGTGCAGT CCAGATCATG GTACTTAGGC AACAGTACCA
AGGCCTTCTG AGCCAAGGAG AAACTGACCT CTAGCCTTCC CAGTTCTAAG 2900
ATTAGAACTA TTAACAAGAC AAGAAGTGGG GAATGAAAGG ATGAAAATGC
AACCTAACCC TCCCAGAACC CAGGAAGTTA ATAAAAAGCT CTAAATGCCC 3000
CCGAATTCCA GACCCTGCTG GCTGCCAGTA AATAGGTAGA AGGTCACACT
TCCTATTGTT CCAGGGCCTG CTATCCTGGC CTAAGTAAGA TAACAGGAAA 3100
TGAGTTGACT AATCGCTTAT CTGGATTCTG TAAAACCGAC TGGCACCATA
GAAGAATTGA TTACACATTG ACAGCCCTAG TGACCTATCT CAACTGCAAT 3200
CTGTCACTCT GCCCAGGAGC CCACGCAGAT GCGGACCTCC GGAGCTATTT
TAAAATGATT GGTCCACGGA GCGCGGGCTC TCGATATTTT AAAATGATTG 3300
GTCCACGGAG CGCGGGCTCT TCGATATTTT AAAATGATTG GTTTGTGACG
CACAGGCTTT GTTGTGAACC CCATAAAAGC TGTCCCGATT CCGCACTCGG 3400
GGCCGCAGTC CTCTACCCCT GCGTGGTGTA CGACTGTGGG CCCCAGCGCG
CTTGGAATAA AAATCCTCTT GCTGTTTGCA TC 3482

SEQ ID NO. 4: Translation of PERV-B *env* (1 letter code)
MHPTLSWRHLPTRGGE PKRLRIPLSFASIAWF LTLTITPQASSKRLID 48
SSNPHRPLSLTWLIID PDTGVTVNSTRGVAPR GTWWPELHFCLRLINP 96
AVKSTPPNLVRSYGFY CCPGTEKEKYCGGSGE SFCRRWSCVTSNDGDW 144
KWPISLQDRVKFSFVN SGPGKYKVMKLYKDKS CSPSDLDYLKISFTEK 192

GKQENIQKWINGMSWG IVFYKYGGGAGSTLTI RLRIETGTEPPVAVGP 240
DKVLAEQGPPALEPPH NLPVPQLTSLRPDITQ PPSNGTTGLIPTNTPR 288
NSPGVPVKTGQRLFSL IQGAFQAINSTDPDAT SSCWLCLSSGPPYYEG 336
MAKEGKFNVTKEHRNQ CTWGSRNKLTLTEVSG KGTCIGKAPPSHQHLC 384
YSTVVYEQASENQYLV PGYNRWWACNTGLTPC VSTSVFNQSKDFCVMV 432
QIVPRVYYHPEEVVLD EYDYRYNRPKREPVSL TLAVMLGLGTAVGVGT 480
GTAALITGPQQLEKGL GELHAAMTEDLRALEE SVSNLEESLTSLSEVV 528
LQNRRGLDLLFLREGG LCAALKEECCFYVDHS GAIRDSMSKLRERLER 576
RRREREADQGWFEGWF NRSPWMTTLLSALTGP LVVLLLLLTVGPCLIN 624
RFVAFVRERVSAVQIM VLRQQYQGLLSQGETD L* 657

SEQ ID NO:5

TGGAAAGATTGGCAACAGCG (SEQ ID NO:5)

SEQ ID NO:6

AGTGATGTTAGGCTCAGTGG (SEQ ID NO:6)

SEQ ID NO:7

TTCTCCTTTGTCAA--TTCCGG 3' (SEQ ID NO:7)

SEQ ID NO:8

TACTTTATCGGGTCCCACTG 3' (SEQ ID NO:8)

SEQ ID NO:9

TACTCTTTTGTTAACAATCCTA 3' (SEQ ID NO:9)

SEQ ID NO:10

TATTCTGAGGCGCGAATAGT 3' (SEQ ID NO:10)

SEQ ID NO:11

ATCCGTCGGCATGCATAATACGACTCAC (SEQ ID NO:11)

SEQ ID NO:12

CGATTCAGTGCTGCTACAAC (SEQ ID NO:12)

SEQ ID NO:13

CCCTTATAACCTCTTGAGCG (SEQ ID NO:13)

SEQ ID NO:14
GTAATGCATGCTTCTATGGTGCCAGTCG (SEQ ID NO:14)

SEQ ID NO:15
CTCTACGCATGCGTGGTGTACGACTGTG (SEQ ID NO:15)

SEQ ID NO:16
GTAATCGGGTCAGACAATGG (SEQ ID NO:16)

CLAIMS

1. An isolated nucleic acid probe, said probe being capable of hybridising to the PERV-B *env* gene under conditions in which said probe is substantially unable to hybridise to the PERV-A *env* gene.

2. An isolated nucleic acid probe according to claim 1 which is capable of hybridising to SEQ ID NO:3 or the complement thereof under conditions in which it is not capable of hybridising to SEQ ID NO:1 or the complement thereof.

3. An isolated nucleic acid probe according to claim 1 or 2 which is derived from the region of PERV-B derived from nucleotides 1000 to 2500 of the SEQ ID NO. 3 isolate.

4. An isolated nucleic acid probe, said probe being capable of hybridising to the PERV-A *env* gene under conditions in which said probe is substantially unable to hybridise to the PERV-B *env* gene.

5. An isolated nucleic acid probe according to claim 4 which is capable of hybridising to SEQ ID NO:1 or the complement thereof under conditions in which it is not capable of hybridising to SEQ ID NO:3 or the complement thereof.

6. An isolated nucleic acid according to claim 4 or 5 which is derived from the region of PERV-A derived from nucleotides 300 to 1809 of the SEQ ID NO:1 isolate.

7. An isolated nucleic acid probe according to any one of the preceding claims which is from 10 to 40 nucleotides in length.

8. A pair of primers suitable for conducting a polymerase chain reaction, at least one of said primers being a nucleic acid as defined in any one of claims 1 to 3.

9. A pair of primers suitable for conducting a polymerase chain reaction, at least one of said primers being a nucleic acid as defined in any one of claims 4 to 6.

10. A method of determining the subtype of a porcine endogenous retrovirus in a sample which contains or is suspected to contain one or both of the PERV-A and PERV-B subtypes, said method comprising probing said tissue with a nucleic acid probe as defined in any one of claims 1 to 7, or by conducting a polymerase chain reaction with a pair of primers as defined in claim 8 or 9, and determining whether or not said probe or pair of primers detects either of said subtypes.

11. A method according to claim 10 wherein retroviral material from said cells is amplified prior to probing or conducting said PCR.

12. A method according to claim 10 wherein the sample is cloned nucleic acid obtained from pig or human cells.

13. A method according to claim 10 or 11 wherein the sample comprises tissue which is primary porcine tissue.

14. A method according to claim 10 or 11 wherein the sample of is a human cell line which has been cultivated in the presence of a porcine cell line.

15. An antibody capable of binding to an epitope on the PERV-B *env* protein under conditions where said antibody is substantially unable to bind to the PERV-A *env* protein.

16. An antibody capable of binding to an epitope on the PERV-A *env* protein under conditions where said antibody is substantially unable to bind to the PERV-B *env* protein.

17. A method of detecting the presence of a pig endogenous

retrovirus in porcine or human tissue or cell lines which comprises bringing a sample of said tissue or cell line into contact with an antibody according to claim 15 or 16 and detecting whether or not said antibody binds to a retrovirus in the sample.

18. Use of a probe according to any one of claims 1 to 7 in a method of determining the subtype of a porcine endogenous retrovirus.

```
                10        20        30        40        50        60
PERV-A  TCGAGTGGGTGAGGCAGCGAGCGTGGAAGCAGCTCCGGGAGGCCTACTCAGGAGGAGACT

                                                           AGA
                                                            |
        700           720                 740               |  760
PERV-B  ................A...........................................

                70        80        90       100       110       120
PERV-A  TGCAAGTTCCACATCGCTTCCAAGTTGGAGATTCAGTCTATGTTAGACGCCACCGTGCAG

                         780                 800                 820
PERV-B  .........................G..................................

               130       140       150       160       170       180
PERV-A  GAAACCTCGAGACTCGGTGGAAGGGACCTTATCTCGTACTTTTGACCACACCAACGGCTG

                         840                 860                 880
PERV-B  .........................C..................................

               190       200       210       220       230       240
PERV-A  TGAAAGTCGAAGGAATCCCCACCTGGATCCATGCATCCCACGTTAAGCCGGCGCCACCTC

                         900                 920                 940
PERV-B  .................T..............................T...........

               250       260       270       280       290       300
PERV-A  CCGATTCGGGGTGGAAAGCCGAAAAGACTGAAAATCCCCTTAAGCTTCGCCTCCATCGCG

                         960                 980                1000
PERV-B  ....C..........G...............G...........................C

               310       320       330       340       350       360
PERV-A  TGGTTCCTTACTCTGTCAATAACTCCTCAAGTTAATGGTAAACGCCTTGTGGACAGCCCG

                        1020                1040                1060
PERV-B  ..............AA..........C..G.CC.G.A...........A.A......T..

               370       380       390       400       410       420
PERV-A  AACTCCCATAAACCCTTATCTCTCACCTGGTTACTTACTGACTCCGGTACAGGTATTAAT

                        1080                1100                1120
PERV-B  ...C......G...T.....C..T......C.GA...T....C.T.A...G...G.C.C.
```

Fig. 1a

```
                 430       440       450       460       470       480
PERV-A    ATTAACAGCACTCAAGGGGAGGCTCCCTTGGGGACCTGGTGGCCTGAATTATATGTCTGC

                          1140                1160                1180
PERV-B    G.A..T.......G...T.TT.....TAGA..C...............C.GC..T.....


                 490       500       510       520       530       540
PERV-A    CTTCGATCAGTAATCCCTGGTCTCAATGACCAGGCCACACCCCCCGATGTACTCCGTGCT

                          1200                      1220
PERV-B    ..C....TGA.T.A...C.C.G.T..--.--.-.-.....T...A.CC..G.....AG.


                 550       560       570       580       590       600
PERV-A    TACGGGTTTTACGTTTGCCCAGGACCCCCAAATAATGAAGAATATTGTGGAAATCCTCAG

            1240                   1260                1280
PERV-B    ..T.....C.:TTGC.........---.A..G.G..A..GA....C.....GGG.T..GG.


                 610       620       630       640       650       660
PERV-A    GATTTCTTTTGCAAGCAATGGAGCTGCATAACTTCTAATGATGGGAATTGGAAATGGCCA

               1300                1320                1340
PERV-B    ..A.C...C..T.G.AG..........G.C..C..C..C.....AG.C...........G


                 670       680       690       700       710       720
PERV-A    GTCTCTCAGCAAGACAGAGTAAGTTACTCTTTTGTTAACAATCCTACCAGTTATAATCAA

                                                                A
                                                                |
               1360                1380                  1400     |
PERV-B    A......TC..G...C.G....AA.T...C.....C..--T...GG..C.GGCA.G....


                 730       740       750       760       770       780
PERV-A    TTTAATTATGGCCATGGGAGATGGAAAGATTGGCAACAGCGGGTACAAAAAGATGTACGA

                        A
                        |
                 1420   |               1440                 1460
PERV-B    AG.G..G.-AA.T..TAA....-A.G..C.GCT.CC..T.-A.ACTT.G.TT..C..-A.


                 790       800       810       820       830       840
PERV-A    AATAAGCAAATAAGCTGTCATTCGTTAGACCTAGATTACTTAAAAATAAGTTTCACTGAA

                                                     GTGG            G
                                                      |              |
                      1480                1500        |   1520       |
PERV-B    G.....--TT.C.-...AA.AAG.AA.ACAGG.A.A..T.C.........A.GGT.-...C
```

Fig. 1b

SUBSTITUTE SHEET (rule 26)

```
                  850       860       870       880       890       900
PERV-A   AAAGGAAAACAAGAAAATATTCAAAAGTGGGTAAATGGTATATCTTGGGGAATAGTGTAC

                             A                                       C
                             |                                       |
                   1540      |         1560                1580      |
PERV-B   TGG....T.GTTTTTT...A.ATGGC.G...AGC.G...CC.CT..AACC.T.C.CT..G

                  910       920       930       940       950       960
PERV-A   TATGGAGGCTCTGGAGAAAGAAAGGATCTGTTCTGACTATTCGCCTCAGAATAGAAACT

                    1600                 1620                1640
PERV-B   G..A...A.---...-..C..-..CCCC....GGCAGTGGGA.C.GAT.A.G..CTGG..

                  970       980       990      1000      1010      1020
PERV-A   CAGATGGAACCTCCGGTTGCTATAGGACCAAATAAGGGTTTGGCCGAACAAGGACCTCCA

                                    C        CTT
                                    |         |
                      1660          |       1680                1700
PERV-B   G.ACA..GG..C....-CC..GG..C...GC.....CCGG..C..C..TT.--.....-.G

                 1030      1040      1050      1060      1070      1080
PERV-A   ATCCAAGAACAGAGGCCATCTCCTAACCCCTCTGATTACAATACAACCTCTGGATCAGTC

                           1720                     1740
PERV-B   C.G.--.GC.T..---...AA.AC.G..G.-..-.--G...CGGT...A......TGA.T

                 1090      1100      1110      1120      1130      1140
PERV-A   CCCACTGAGCCTAACATCACTATTAAAACAGGGGCGAAACTTTTTAGCCTCATCCAGGGA

                CAC AGA TCCCCAGGT
                 |   |   |
         1760    |   |   |              1800                1820
PERV-B   ..T..CA........G.TC..G....G.....ACA..G...C..C..T............

                 1150      1160      1170      1180      1190      1200
PERV-A   GCTTTTCAAGCTCTTAACTCCACGACTCCAGAGGCTACCTCTTCTTGTTGGCTTTGCTTA

                                 C
                                 |
                1840             |  1860                1880
PERV-B   .....C.....CA.C...........-..T..T..C..T................TC..

                 1210      1220      1230      1240      1250      1260
PERV-A   GCTTCGGGCCCACCTTACTATGAGGGAATGGCTAGAGGAGGGAAATTCAATGTGACAAAG

                1900                1920                1940
PERV-B   T.C..A..G..T.....T........G.......A..A...A..............C..A
```

Fig. 1c

```
                1270      1280      1290      1300      1310      1320
PERV-A    GAACATAGAGACCAATGTACATGGGGATCCCAAAATAAGCTTACCCTTACTGAGGTTTCT

              1960                1980                2000
PERV-B    ..G......A.T..............G....G...............C.....A.....C


                1330      1340      1350      1360      1370      1380
PERV-A    GGAAAAGGCACCTGCATAGGGATGGTTCCCCCATCCCACCAACACCTTTGTAACCACACT

              2020                2040                2060
PERV-B    ..G..G..G..A........A.AA.C.......................CT.TAGT...


                1390      1400      1410      1420      1430      1440
PERV-A    GAAGCCTTTAATCGAACCTCTGAGAGTCAATATCTGGTACCTGGTTATGACAGGTGGTGG

              2080                2100                2120
PERV-B    .TG.TT.A.G.G.AGG....A..A.A...G...T.A.............A...........


                1450      1460      1470      1480      1490      1500
PERV-A    GCATGTAATACTGGATTAACCCCTTGTGTTTCCACCTTGGTTTTCAACCAAACTAAAGAC

              2140                2160                2180
PERV-B    .....C........G........C..............CA..C.........T.C.....T


                1510      1520      1530      1540      1550      1560
PERV-A    TTTTGCGTTATGGTCCAAATTGTCCCCCGGGTGTACTACTATCCCGAAAAAGCAGTCCTT

              2200                2220                2240
PERV-B    ..C..T..C...........C........A.........C....T..GG...TG......


                1570      1580      1590      1600      1610      1620
PERV-A    GATGAATATGACTATAGATATAATCGGCCAAAAAGAGAGCCCATATCCCTGACACTAGCT

              2260                2280                2300
PERV-B    ...............C.G.....C..A...........A...G.......T..C......


                1630      1640      1650      1660      1670      1680
PERV-A    GTAATGCTCGGATTGGGAGTGGCTGCAGGCGTGGGAACAGGAACGGCTGCCCTAATCACA

              2320                2340                2360
PERV-B    ..............A..GAC...C.TT.....A........G..A........G......


                1690      1700      1710      1720      1730      1740
PERV-A    GGACCGCAACAGCTGGAGAAAGGACTTAGTAACCTACATCGAATTGTAACGGAAGATCTC

              2380                2400                2420
PERV-B    .....A..G.....A............G..G.G......GCGGCCA.G..A.........
```

Fig. 1d

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                 1750      1760      1770      1780      1790      1800
PERV-A  CAAGCCCTAGAAAAATCTGTCAGTAACCTGGAGGAATCCCTAACCTCCTTATCTGAAGTG

              2440                2460                2480
PERV-B  .G....T....GG.G.....T..C.....A..A..G.....G..T..T..G.........

                 1810      1820      1830      1840      1850      1860
PERV-A  GTTCTACAGAACAGAAGGGGGTTAGATCTGTTATTTCTAAAAGAAGGAGGGTTATGTGTA

              2500                2520                2540
PERV-B  ............C.G....A.........C.G.......G......T...........C.

                 1870      1880      1890      1900      1910      1920
PERV-A  GCCTTAAAAGAGGAATGCTGCTTCTATGTAGATCACTCAGGAGCCATCAGAGACTCCATG

              2560                2580                2600
PERV-B  ...........A....T...........................................

                 1930      1940      1950      1960      1970      1980
PERV-A  AGCAAGCTTAGAGAAAGGTTAGAGAGGCGTCGAAGGGAAAGAGAGGCTGACCAGGGGTGG

              2620                2640                2660
PERV-B  ............................................................

                 1990      2000      2010      2020      2030      2040
PERV-A  TTTGAAGGATGGTTCAACAGGTCTCCTTGGATGACCACCCTGCTTTCTGCTCTGACGGGG

              2680                2700                2720
PERV-B  ...........................................................A

                 2050      2060      2070      2080      2090      2100
PERV-A  CCCCTAGTAGTCCTGCTCCTGTTACTTACAGTTGGGCCTTGCTTAATTAATAGGTTTGTT

              2740                2760                2780
PERV-B  ............................................................

                 2110      2120      2130      2140      2150      2160
PERV-A  GCCTTTGTTAGAGAACGAGTGAGTGCAGTCCAGATCATGGTACTTAGGCAACAGTACCAA

              2800                2820                2840
PERV-B  ............................................................

                 2170      2180      2190      2200      2210      2220
PERV-A  GGCCTTCTGAGCCAAGGAGAAACTGACCTCTAGCCTTCCCAGTTCTAAGATTAGAACTAT

              2860                2880                2900
PERV-B  ............................................................
```

Fig. 1e

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              2230      2240      2250      2260      2270      2280
PERV-A   TAACAAGACAAGAAGTGGGAATGAAAGGATGAAAATGCAACCTAACCCTCCCAGAACCC

             2920                2940                2960
PERV-B   ............................................................

              2290      2300      2310      2320      2330      2340
PERV-A   AGGAAGTTAATAAAAAGCTCTAAATGCCCCCGAATTCCAGACCCTGCTGGCTGCCAGTAA

             2980                3000                3020
PERV-B   ............................................................

              2350      2360      2370      2380      2390      2400
PERV-A   ATAGGTAGAAGGTCACACTTCCTATTGTTCCAGGGCCTGCTATCCTGGCCTAAGTAAGAT

             3040                3060                3080
PERV-B   ............................................................

              2410      2420      2430      2440      2450      2460
PERV-A   AACAGGAAATGAGTTGACTAATCGCTTATCTGGATTCTGTAAAACCGACTGGCACCATAG

             3100                3120                3140
PERV-B   ............................................................
```

Fig. 1f

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(57) Abstract

The present invention is based upon the finding that porcine endogenous retroviruses exist in two different subtypes, which we have termed PERV-A and PERV-B. The differences are reflected in sequence divergence in the envelope genes, and these differences may be used to provide nucleic acid and antibody probes which can distinguish between the two subtypes. This allows patterns of subtype transmission between cells, particularly porcine to human cells, to be monitored.

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INTERNATIONAL SEARCH REPORT

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B. FIELDS SEARCHED

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IPC 6 C12Q C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	LE TISSIER ET AL: "Two sets of human-tropic pig virus" NATURE, vol. 389, October 1998, pages 681-82, XP002084123 see the whole document ---	1-18
P,A	WO 97 40167 A (Q ONE BIOTECH LTD ;IMUTRAN LTD (GB); GALBRAITH DANIEL NORMAN (GB);) 30 October 1997 see the whole document ---	1-18
P,A	WO 97 21836 A (GEN HOSPITAL CORP) 19 June 1997 see the whole document ---	1-18
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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A	STOVE J P ET AL: "THE DANGERS OF XENOTRANSPLANTATION" NATURE MEDICINE, vol. 1, no. 11, November 1995, page 1100 XP002037073	

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